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Hair Growth Modulation by Topical Immunophilin Ligands

Induction of Anagen, Inhibition of Massive Catagen Development, and Relative Protection from Chemotherapy-Induced Alopecia

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Selected immunophilin ligands (IPLs) are not only potent immunosuppressants but also modulate hair growth. Their considerable side effects, however, justify at best topical applications of these drugs for the management of clinical hair growth disorders. Therefore, we have explored hair growth manipulation by topical cyclosporin A (CsA) and FK 506 in previously established murine models that mimic premature hair follicle regression (catagen) or chemotherapy-induced alopecia, two major pathomechanisms underlying human hair loss. We confirm that topical CsA and FK 506 induce active hair growth (anagen) in the back skin of C57BL/6 mice with all follicles in the resting stage (telogen) and show that both IPLs also inhibit massive, dexamethasone-induced, premature catagen development in these mice. Furthermore, we demonstrate that CsA and FK 506 provide relative protection from alopecia and follicle dystrophy induced by cyclophosphamide, possibly by favoring the dystrophic anagen pathway of follicle response to chemical damage. Although it remains to be established whether these IPLs exert the same effects on human hair follicles, our study provides proof of the principle that topical IPLs can act as potent manipulators of clinically relevant hair-cycling pathomechanisms. This strongly encourages one to explore the use of topical IPLs in the management of human hair growth disorders. (Am J Pathol 1997, 150:1433–1441)

Immunosuppressive immunophilin ligands (IPLs) such as cyclosporin A (CsA) and FK 506 are now appreciated as potent hair growth modulatory agents in rodents and/or man, namely, as inducers of active hair growth (anagen)[1–5] and as inhibitors of hair follicle regression (catagen).[6–8] As most cases of hair loss seen in clinical practice essentially represent disturbances of hair follicle cycling that are based on a premature termination of anagen and induction of catagen,[6,9] it is of significant clinical interest to explore the potential catagen-inhibitory properties of IPLs under clinically relevant experimental conditions.

Previously, we have developed two mouse models for the induction and pharmacological manipulation of normal, but premature, massive catagen development[6] and for chemotherapy-induced alopecia.[7] These models simulate the two major pathomechanisms underlying the development of alopecia in man: hair loss caused primarily due to premature anagen termination (eg, telogen effluvium, androgenetic alopecia, and diffuse alopecia due to endocrine abnormalities) and hair loss based on the induction of follicle dystrophy usually in conjunction with premature anagen termination (eg, alopecia areata and chemotherapy-induced alopecia).[9] Using these models, we could show that high doses of intraperitoneally administered CsA significantly inhibit both spontaneous catagen development[8] as

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well as pharmacologically induced, massive, and premature catagen development in mice.[6] In addition, we have determined that high doses of intraperitoneally administered CsA provide relative protection from chemotherapy-induced follicle dystrophy in mice and favor the so-called dystrophic anagen pathway of anagen follicles responding to cyclophosphamide damage over the so-called dystrophic catagen pathway.[7]

However, due to their considerable side effects,[10] clinical use of these IPLs for treating hair growth disorders appears justifiable only if efficacy upon topical administration in clinically relevant animal models can be demonstrated. In the current studies, we have, therefore, explored whether topical CsA or FK 506 inhibit massive premature catagen development and chemotherapy-induced alopecia and follicle dystrophy in our previously described C57BL/6 mouse model for hair research.[3,6–8,11]

Mice with all back skin hair follicles in the anagen stage of the hair cycle were treated with either topical dexamethasone-21-acetate (DEX) for triggering premature catagen development or intraperitoneal cyclophosphamide (CYP) to induce alopecia and follicle dystrophy. A 0.01% to 0.5% concentration of CsA or 0.01% to 0.5% concentration of FK 506 or vehicle was co-administered to the back skin of these mice for 5 or 7 days. Test and control mice were compared with respect to the time of catagen development (macroscopic: skin color conversion from black to pink, declining skin thickness; microscopic: histomorphometry of hair cycle stages), alopecia and/or follicle dystrophy (cf Refs. 6 and 7). For comparison and as a positive control, the tested doses of CsA and FK 506, using 100% ethanol as vehicle, were probed with respect to their well appreciated anagen-inducing properties in mice.[1–5]

Materials and Methods

Animals

Six- to nine-week-old, female C57BL/6 mice (Charles River, Sulzfeld, Germany) were housed in groups of 10 to 12 animals in polycarbonate cages (type III, UNO, The Netherlands) under conventional standardized conditions (21 ± 1°C, 55 ± 5% humidity, 10 changes of fresh filtered air per hour, 12/12-hour light (150 Lux)/dark cycle). Animals had free access to water and mouse chow. Mice were in the telogen stage of the hair cycle when used (ie, pink skin color[6,7,11,12]).

Anagen Induction by Topical CsA or FK 506

As all mature melanocytes in truncal skin of C57BL/6 mice are confined to the hair follicles and as melanogenesis is strictly coupled to the growth phase of the hair cycle (anagen), the increase in back skin pigmentation during anagen development results in a controlled and highly reproducible, macroscopically appreciable change of skin color from pink (telogen skin) to gray (early anagen) to black (late anagen).[11–13] Therefore, the change of back skin color in C57BL/6 mice is a reliable marker for the differentiation between skin in the resting phase of the hair cycle (telogen) and anagen.

In two separate experiments, a total of 105 mice (31 control, and 74 test) with all back skin hair follicles in telogen as judged by their homogeneously pink back skin color were shaved carefully with an electrical clipper (Minicut, Wella, Darmstadt, Germany). With the help of a pipette, 150 μl of CsA (Sandimmun, Sandoz, Basel, Switzerland; 0.5% (4.2 mmol/L), 0.1% (0.8 mmol/L), or 0.01% (0.08 mmol/L)), FK 506 (Fujisawa Pharmaceutical Co., Osaka, Japan; 0.5% (6.2 mmol/L), 0.1% (1.24 mmol/L), or 0.01% (0.12 mmol/L)) in ethanol, or vehicle as control, were evenly applied to the back skin for 9 days once daily. The back skin color of all animals was recorded daily up to 18 days after onset of treatment and the number of mice in telogen (pink skin) or anagen (gray/black skin) was documented and expressed as the percentage of animals in anagen (cf Refs. 1, 3, and 14).

Inhibition of Dexamethasone-Induced Catagen by Topical CsA or FK 506

Mice in the telogen stage of the hair cycle were induced to enter anagen by depilation as previously described.[13] Briefly, a melted wax/rosin mixture was applied to the back skin and peeled off after hardening, thus plucking all telogen hair shafts, which invariably induces the development of mature, hair-shaft-producing anagen VI hair follicles within 9 days after depilation. This process is preceded by a marked increase in skin pigmentation and thickness by days 5 to 6 after depilation[13,15] and results in the visible appearance of uniformly black skin hair shafts within 11 to 12 days after anagen induction. These depilation-induced anagen follicles are macroscopically, histologically, and functionally indistinguishable from spontaneously cycling hair follicles.[15] At the end of anagen VI (ie, 17 to 20 days after anagen induction), spontaneously occurring follicle regression (catagen) terminates the depilation-induced hair cycle, which is associated with and heralded by

reconversion of the skin color from black to gray to pink.[6,11,12]

Starting on day 9 after depilation, when back skin hair follicles had just entered anagen VI, 1 ml of 0.1% DEX in propylene glycol was administered once daily with a paintbrush to the back skin of mice for 5 days. This treatment results in the induction of macroscopically and microscopically normal but unusually massive and premature termination of anagen (ie, catagen induction) within the entire treated skin area as soon as on day 13 after depilation, thus yielding large, homogeneous back skin regions in defined stages of the anagen-catagen-telogen transition of the murine hair cycle.[6]

Three different concentrations of both CsA and FK 506 (0.5%, 0.1%, or 0.01%; 150 μl) or vehicle as control (ethanol) were applied once daily to the back skin of DEX-treated mice for seven days with the help of a pipette starting on day 7 after anagen induction by depilation. Either 10 or 12 animals per group were used in two experiments (104 in total: 22 control and 82 test animals). On day 16, hair follicle regression was assessed macroscopically by documentation of skin pigmentation. The relative extension of the back skin area showing color conversion from black to gray-pink was assessed and recorded independently by two investigators and was documented as percentage of total back skin area not showing catagen-associated depigmentation (cf Refs. 6 and 11).

Histology

All macroscopic observations were controlled, verified, and quantified by histology as previously described.[6,7] Formalin-fixed, paraffin-embedded, and Giemsa-stained skin sections were taken from standardized back skin regions on day 16 after depilation and were analyzed by standardized histomorphometry.[6,7] A minimum of 50 back skin hair follicles per mouse were assigned to the hair cycle stages anagen (ana VI), early catagen (cat I-III), mid-catagen (cat IV-V), and late catagen (cat VI-VIII) according to Chase[16] and Straile et al[17] by their characteristic, stage-specific histological appearance. The percentage of follicles in each defined hair cycle stage was calculated. Hair cycle score (HCS) values of individual mice were obtained by multiplication of the percentages of hair follicles in these defined hair cycle stages (according to a score of 0 for anagen VI, 1 for early catagen, 2 for mid-catagen, and 3 for late catagen) and subsequent addition of the resulting values (for example, if 100 follicles of mouse A were analyzed and 50% were assigned to anagen VI, 30% to early catagen, and 20% to mid-catagen, then [50 × 0] + [30 × 1] + [20 × 2] = HCS of 70). Mean and standard error of the mean (SEM) were calculated on pooled data, derived from at least 10 different mice per test or control group, studied in two separate experiments. The level of significance was calculated by the independent Student's *t*-test for unpaired samples.

Inhibition of Cyclophosphamide-Induced Dystrophic Catagen by CsA or FK 506

A single intraperitoneal injection of CYP (150 mg/kg body weight in distilled water) was given to mice in the anagen stage of the hair cycle, 9 days after its induction by depilation, as described.[7] This treatment causes severe alopecia, based on follicle dystrophy and pathological, premature termination of anagen,[7] along with severe disruption of the follicular pigmentation apparatus.[18]

CYP-injected animals were treated with daily topical applications of CsA or FK 506 (at 0.5%, 0.1%, or 0.01%; 150 μl) or vehicle as control from days 7 to 11 after anagen induction by depilation. There were 10 mice per test and control group; a total of 70 (10 control and 60 test) animals was studied. All mice were examined daily until day 19 for signs of alopecia and palpable reduction of skin thickness. Alopecia was documented as the percentage of back skin surface showing signs of hair loss, and skin thickness was quantified with a cutimeter (7309 (0.01 to 9 mm), Mitutoyo, Japan) on day 19 after depilation.

Histology

Back skin was collected for histomorphometric analysis on day 19. On Giemsa-stained, longitudinal sections, at least 50 follicles per mouse were classified according to their hair cycle stage, following the criteria of Chase,[16] Straile et al,[17] Paus et al,[7] and Slominski et al.[18] The percentage of follicles in dystrophic anagen VI, dystrophic early, mid-, or late catagen was calculated and multiplied with the following stage values: 0, dystrophic anagen VI; 1, early dystrophic catagen; 2, mid-dystrophic catagen; 3, late dystrophic catagen. Progression of individual mice in the hair cycle was then assessed by addition of all values and expressed as mean ± 1 SEM of this score.

Furthermore, follicles were screened and scored for signs of protection from CYP-induced dystrophy[7,18] applying the following criteria: 0, normal follicles, no signs of dystrophy; 1, one to five ectopic melanin granules; 2, more than five ectopic melanin

granules; 3, extrusion of less than five 5 melanin granules into the perifollicular dermis, swelling of dermal papilla; 4, large groups of perifollicular melanin granules. Data were derived from analyzing a minimum of 20 follicles per section from at least 10 mice per group and were expressed as mean of score ± 1 SEM. *P* values were calculated employing the independent Student's *t*-test for unpaired samples.

Results

Topical CsA and FK 506 Induce Active Hair Growth (Anagen)

Anagen was induced by both compounds. The topical hair growth induction activity of CsA was restricted to the treated skin area and was concentration dependent; at 0.5% CsA, anagen development had occurred in all animals by day 15 and at 0.1% by day 18. At the end of the observation (18 days after onset of treatment), the percentage of mice in anagen was 80% and 50% in animals treated with 0.01% CsA and the vehicle, respectively (Figure 1A). (This significantly retarded, but still high, anagen induction rate after prolonged vehicle treatment is presumably due to the irritant properties of ethanol, which are well recognized to suffice as anagen induction stimulus.[3]) Different from CsA, the hair growth induction activity of FK 506 did not show clear dose dependency. By day 17, anagen induction was observed in 90% of the mice treated with 0.1% FK 506, whereas in only 33% of the animals treated with 0.5% and in 55% of the animals treated with 0.01% (Figure 1B).

Topical CsA and FK 506 Inhibit Massive, Pharmacologically Induced Catagen

Assessment of the skin area showing catagen-associated color conversion from black to gray-pink revealed significant inhibition of DEX-induced catagen by topical application of CsA and FK 506. Again, the activity of CsA was concentration dependent, and at 0.5%, was higher than that of FK 506, which was similarly efficient at 0.5% and 0.1%, equaling CsA at 0.1% (Figure 2A). As seen in Figure 2B, animals treated with 0.5% CsA exhibited the anagen-specific, gray-black back skin color, and less than 20% of the treated skin area (calculated from all animals) was in catagen as compared with 75% in vehicle-treated animals ($P < 0.001$; Figure 2A). Treatment with 0.1% CsA or with FK 506 (0.1% or 0.5%) caused inhibition of catagen-associated depigmentation in

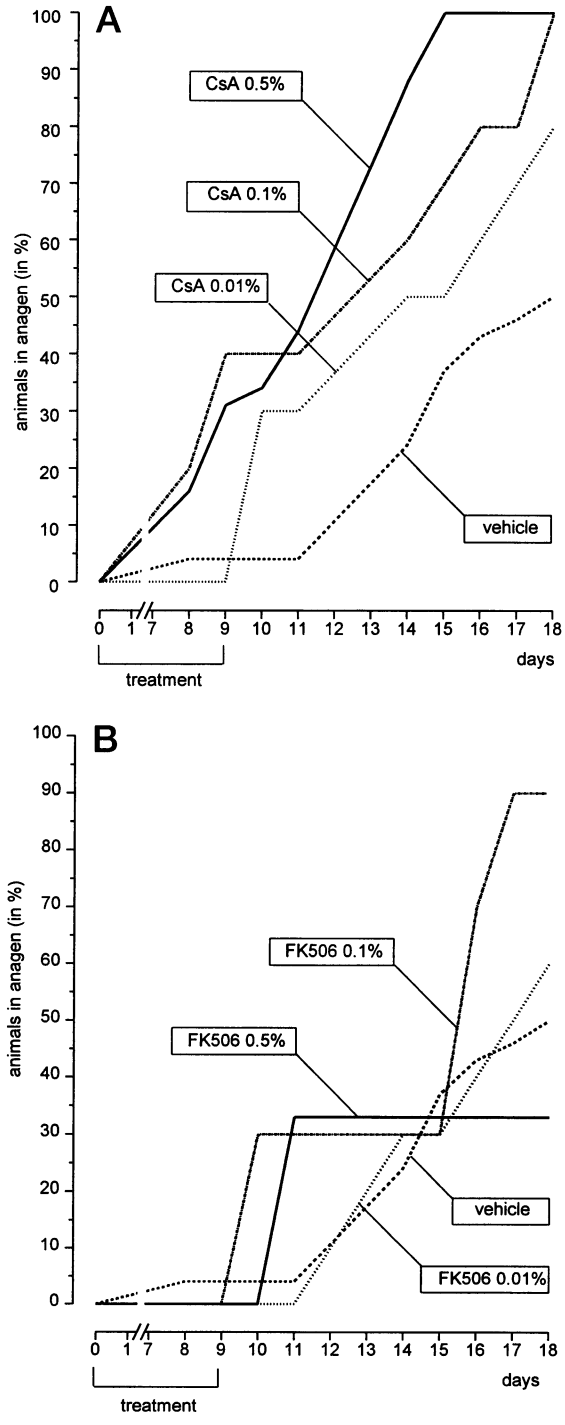

Figure 1. *Topical CsA and FK 506 induce anagen.* A: *Number of days after start of topical treatment with different concentrations of CsA is shown on the x axis.* B: *Number of days after start of topical treatment with different concentrations of FK 506 is shown on the x axis.* A *and* B: *The percentage of animals showing change of back skin color from pink (telogen) to gray/black (anagen) is shown on the y axis. Data are from two separate experiments with a total of 10 to 22 animals per group.*

55 to 65% of the treated skin area (Figure 2A). Whereas 8 of 22 animals treated with vehicle (controls) showed back skin that entirely resembled telo-

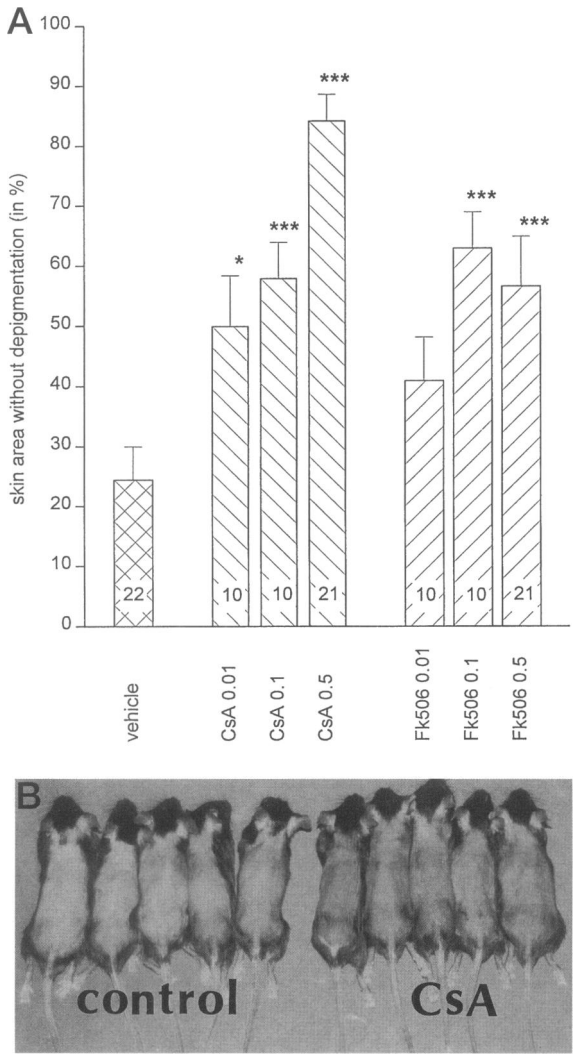

Figure 2. *CsA and FK 506 inhibit DEX-induced catagen: macroscopic effect.* **A:** *The x axis shows concentrations in percent of CsA or FK 506 used for topical treatment of back skin with catagen-induced by DEX. The y axis shows the mean (percent) ± 1 SEM of total back skin area on day 16 after anagen induction by depilation in percent of total back skin area not showing catagen-associated depigmentation. Data are from two separate experiments with a total of 10 to 21 animals per group.* ***$P \leq 0.001$; *$P \leq 0.05$. **B:** *Representative C57BL/6 mice on day 16 after anagen induction by depilation that have received DEX on days 9 to 13 after depilation. Mice were treated topically with 0.5% CsA or vehicle (days 7 to 13) and shaved to demonstrate the black back skin of mice treated with CsA as opposed to the pink-gray skin color of vehicle-treated mice (indicating rapid, extensive, and premature induction of a homogeneous catagen wave after treatment with DEX).*

gen, only 4 of 21 animals treated with 0.5% FK 506 and none of the animals treated with 0.5% CsA had completely pink back skin. This corresponded to 71 and 43% of animals with more than 80% pigmented skin after application of 0.5% CsA and 0.5% FK 506, respectively, as compared with 0% in the control group. At 0.01% CsA or FK 506, catagen was significantly inhibited only by CsA.

These macroscopic observations corresponded to a significant retardation of DEX-induced catagen progression of CsA- or FK-506-treated mice, as verified by histomorphometry. Both CsA and FK 506 caused highly significant inhibition of DEX-induced follicle regression (Figure 3, A and B). When mice were scored on day 16 after the induction of anagen by depilation for progression of hair follicles in the hair cycle (hair cycle score), DEX-treated control animals averaged close to 200 score points, corresponding to mid-catagen. CsA- and FK-506-treated mice showed scores below 100, which is representative of the transition of anagen VI toward early catagen. CsA was most effective in the highest concentration tested (0.5%), whereas FK 506 showed best retardation of catagen development at the lowest concentration (0.01%).

Topical CsA and FK 506 Modulate/Retard Chemotherapy-Induced Alopecia and Dystrophic Follicle Regression

Both CsA and FK 506 caused significant protection from CYP-induced alopecia (Figures 4 to 6). On day 14 after depilation, topical treatment with CsA resulted in a modest decrease and external application of FK 506 in a marked reduction of the alopecic skin area after CYP treatment (Figure 6, representative test and control mice shown). As seen in Figure 4, the extension of the back skin area affected by CYP-induced anagen effluvium was significantly smaller than in controls when mice were treated with topical FK 506 or CsA (at both 0.5% and 0.1%). Maximal reduction of alopecia to less than one-half of the back skin area was seen with 0.5% FK 506. Although no substantial differences between test and control groups were seen regarding skin color changes on day 16 after depilation, both CsA and FK 506 also significantly inhibited the decrease in skin thickness that occurs due to the induction of (dystrophic) catagen by CYP[7] (Figure 5). This indicated that a higher percentage of CYP-treated anagen VI follicles had remained in dystrophic anagen when CsA or FK 506 were co-administered with CYP, as opposed to the relatively high percentage of dystrophic catagen follicles in mice treated only with CYP and topical vehicle (cf Refs. 7 and 14).

Histomorphometric analysis (Figures 7 and 9) revealed a highly significant retardation of follicle progression toward dystrophic catagen in the test groups. When scored for the progression from anagen *via* catagen toward telogen (hair cycle score), all follicles in CYP-treated animals had reached the late

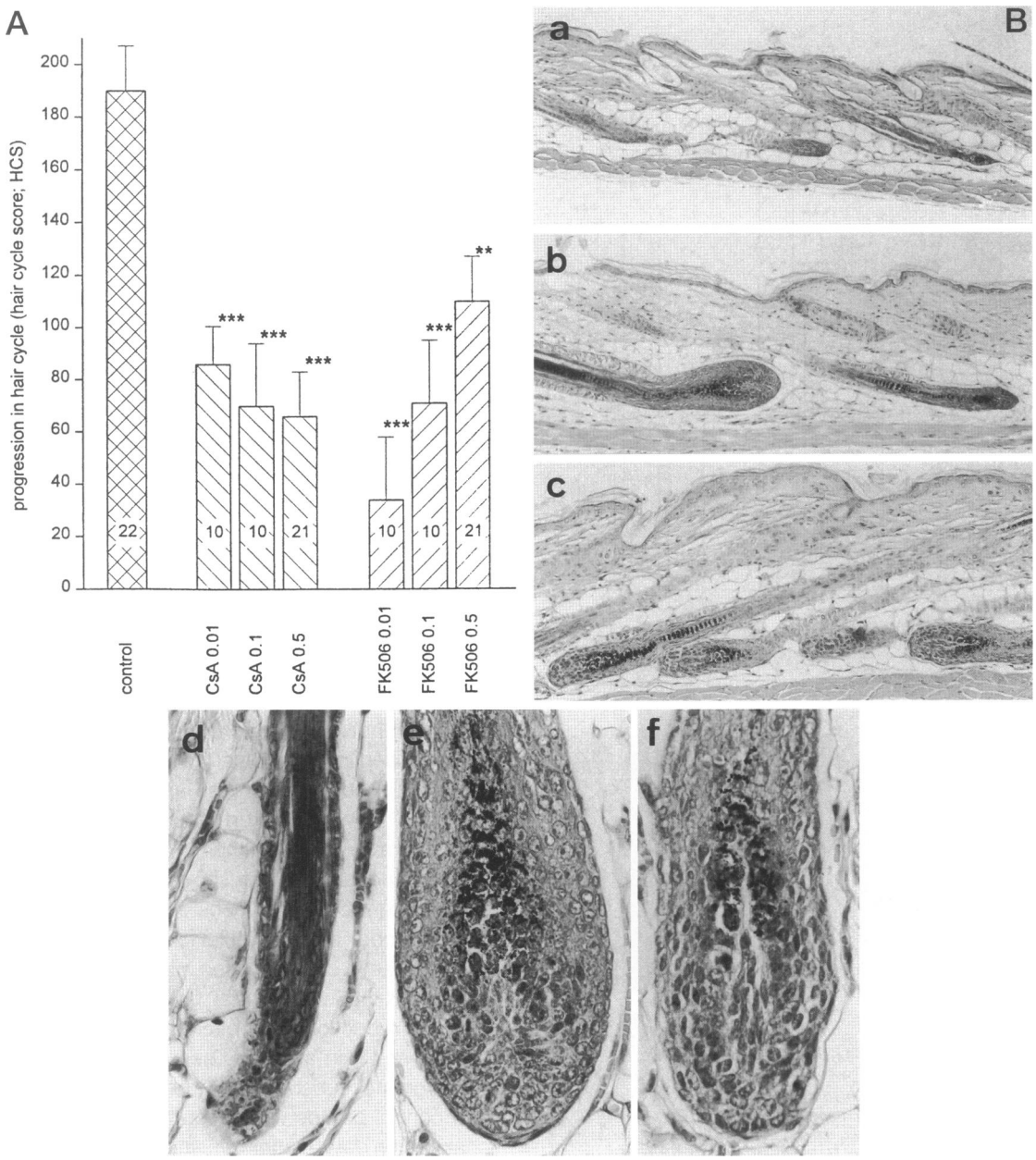

Figure 3. *CsA and FK 506 inhibit DEX-induced catagen: microscopic effects.* **A:** *On the x axis are concentrations of CsA or FK 506 in percent used for topical treatment of back skin with DEX-induced catagen. On the y axis is the mean ± 1 SEM of the histomorphometric score (Hair cycle score, HCS) assessed on day 16 after anagen induction. For every mouse, a minimum of 50 individual follicles was assigned to defined hair cycle stages (anagen VI, early catagen (CAT I to III), mid-catagen (CAT IV and V), and late catagen (CAT VI to VIII)), and the percentage of follicles in these cycle stages was determined. Score was calculated by multiplication of the percentage of the hair cycle stages with its assigned value (0 = anagen, 1 = early catagen, 2 = mid-catagen, and 3 = late catagen). For a detailed explanation of HCS, see Materials and Methods). Data are from two separate experiments with a total of 10 to 21 animals per group.* ***$P \leq 0.001$; **$P \leq 0.005$. **B:** *Histological appearance of DEX-treated mouse skin on day 16 after anagen induction.* **a:** *DEX treatment alone shows follicles that have entered late stages of follicle regression. Giemsa; magnification, ×400.* **b:** *Follicles in back skin treated with topical CsA remain mostly in anagen VI.* **c:** *Skin treated with FK 506 shows follicles in the anagen stage of the hair cycle.* **d:** *Representative catagen follicle after DEX treatment. Magnification, ×1000).* **e** *and* **f:** *Representative follicles in the anagen VI stage of the hair cycle after treatment with 0.5% CsA or 0.5% FK 506.*

stages of catagen (>250 score points), whereas treatment with effective concentrations of CsA (0.1 and 0.5%) or FK 506 (0.01 to 0.5%) caused a significant retardation of follicle regression with hair cycle scores representative of mid-catagen (Figure 7).

Topical CsA and FK 506 provided relative protection from chemotherapy-induced follicle dystrophy. Assessing follicle morphology with regard to signs of CYP-induced dystrophy as judged mainly by characteristic, CYP-induced disruptions of the follicle

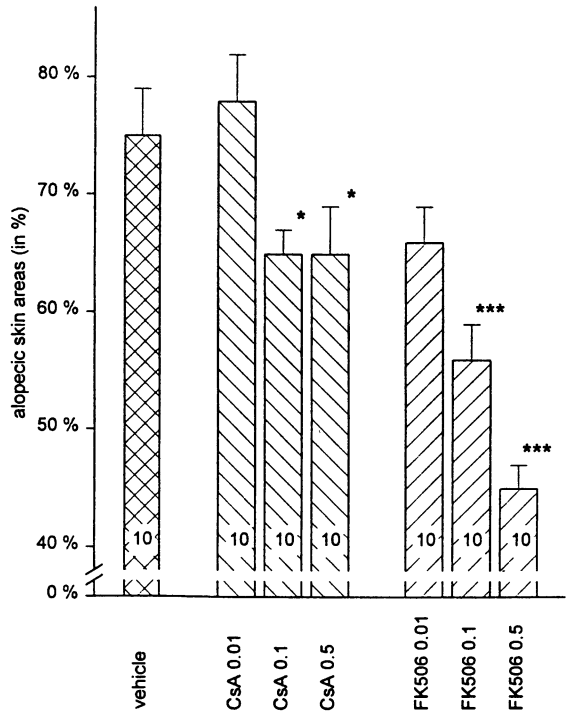

Figure 4. *CsA and FK 506 inhibit CYP-induced alopecia. On the x axis are concentrations of CsA or FK 506 used for topical treatment of back skin after induction of dystrophic catagen by CYP. On the y axis, bars represent mean ± 1 SEM of the back skin area on day 14 affected by alopecia after application of CYP. A total of 10 mice per group were tested. ***P ≤ 0.001; *P ≤ 0.05.*

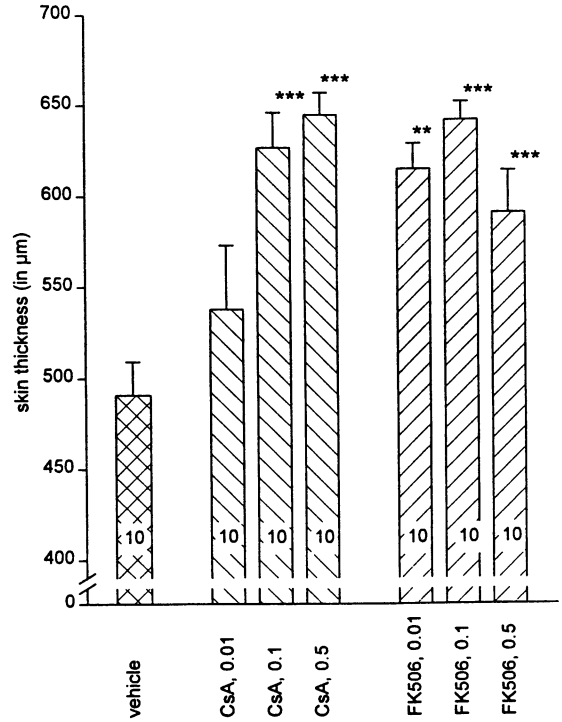

Figure 5. *CsA and FK 506 inhibit CYP-induced dystrophic catagen as assessed by measuring skin-fold thickness. On the x axis are concentrations of CsA or FK 506 used for topical treatment of back skin after induction of dystrophic catagen by CYP. On the y axis is the mean ± 1 SEM of skin thickness measured at the test site on day 19 after anagen induction by depilation. Ten mice per group were tested. ***P ≤ 0.001; **P ≤ 0.005.*

pigmentation apparatus,[18] both CsA and FK 506 exhibited relative protection from CYP-induced follicle damage (Figure 8), even though CYP-induced alopecia did eventually develop in all test and control mice. Most notably, the topical application of 0.1 or 0.5% CsA and 0.1% FK 506 led to a highly significant reduction of ectopically located melanin granules and melanin clumping, as shown in representative hair follicles in Figure 9.

Discussion

Here we provide convincing evidence that the topical application of two immunosuppressive IPLs, CsA and FK 506, not only induces anagen in mice (Figure 1, A and B), as previously demonstrated by ourselves and others,[1–5] but also significantly inhibits massive, premature catagen development (Figures 2 and 3) as well as chemotherapy-induced alopecia and follicle dystrophy (Figures 4 to 9). This corresponds very well to our previous finding that intraperitoneally injected CsA exerts the same effects.[6,7] That these results are due to the topical action of IPLs rather than systemic mechanisms is supported by earlier observations showing localized hair-cycle-modulating effects of topical CsA limited to the skin area of application.[3]

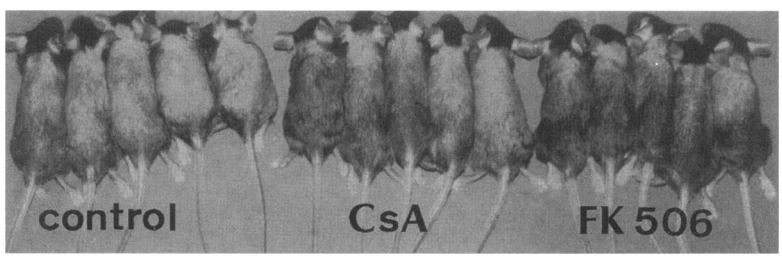

Figure 6. *CsA and FK 506 inhibit CYP-induced dystrophic catagen: macroscopic effects. Representative mice are shown on day 14 after depilation after treatment with CYP (day 9) and application of 0.5% FK 506, 0.5% CsA, or vehicle (from days 7 to 11 after depilation). Note that mice in the vehicle-treated group show significantly greater areas of back skin affected by alopecia as compared with FK-506- and CsA-treated animals.*

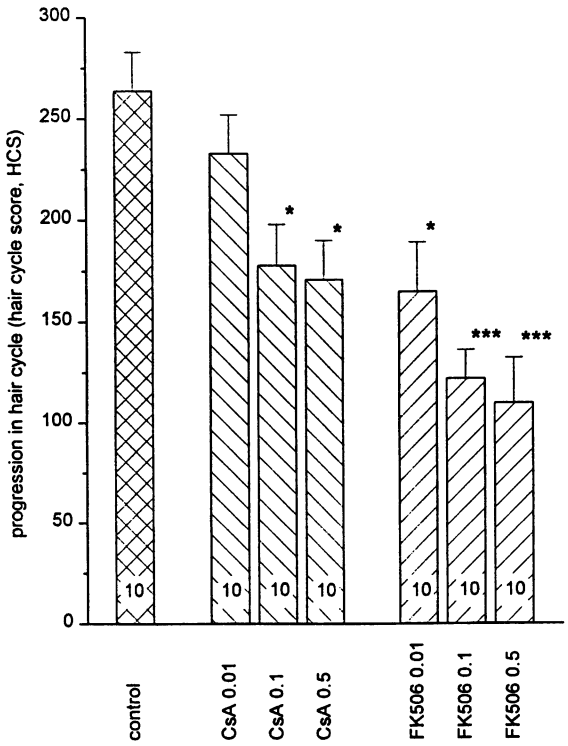

Figure 7. *Protection from CYP-induced progression toward dystrophic catagen by CsA and FK 506. On the x axis are concentrations (in percent) of CsA or FK 506 used for topical treatment of back skin after induction of dystrophic catagen by CYP. On the y axis is the mean ± 1 SEM of histomorphometric score (hair cycle score, HCS) assessed on day 19 after anagen induction. For every mouse a minimum of 50 individual follicles was assigned to defined hair cycle stages (anagen VI, early catagen (CAT I to III), mid-catagen (CAT IV and V), or late catagen (CAT VI to VIII), and the percentage of follicles in these cycle stages was determined. Score was calculated by multiplication of the percentage of the these hair cycle stages with its assigned value (0 = anagen, 1 = early catagen, 2 = mid-catagen, and 3 = late catagen; for detailed explanation of HCS, see Materials and Methods). Ten animals per group were examined. ***P ≤ 0.001; *P ≤ 0.05*

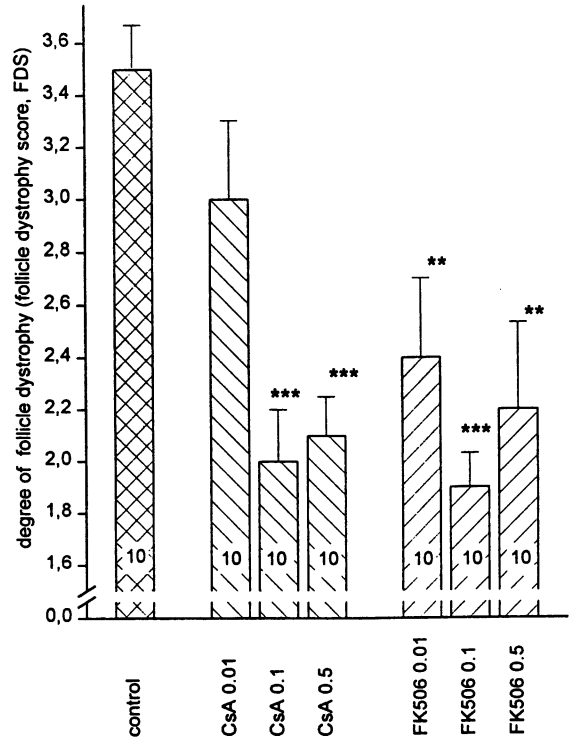

Figure 8. *Protection from CYP-induced follicle dystrophy by CsA and FK 506. On the x axis are concentrations of CsA or FK 506 used for topical treatment of back skin after induction of dystrophic catagen by CYP. On the y axis is the mean ± 1 SEM of follicle dystrophy score assessed on day 19 after anagen induction. A minimum of 20 follicles per mouse were scored according to signs of follicle dystrophy: 0, normal follicles, no signs of dystrophy; 1, one to five ectopic melanin granules; 2, more than five ectopic melanin granules; 3, extrusion of less than five melanin granules into the perifollicular dermis, swelling of dermal papilla; 4, large groups of perifollicular melanin granules. Ten animals per group were examined. ***P ≤ 0.001; **P ≤ 0.005.*

Although it remains to be explored whether similar hair growth modulatory effects by CsA and FK 506 can also be obtained in the human system, these studies demonstrate for the first time that it is feasible, in principle, to inhibit catagen, alopecia, and follicle dystrophy by topical IPL application. In addition, this proof of principle was generated using two animal models that simulate clinically relevant hair cycling pathomechanisms more closely than any other currently available animal model.[6,7,14] Therefore, it is reasonable to systematically explore whether topical IPLs with a favorable skin permeation profile are also effective agents for the therapeutic management of human hair growth disorders like telogen effluvium, androgenetic alopecia, alopecia areata, and chemotherapy-induced alopecia (cf Ref. 9).

Alternative to using IPLs, the underlying signal transduction pathways of IPL-induced changes in hair follicle cycling might be directly targeted by appropriate

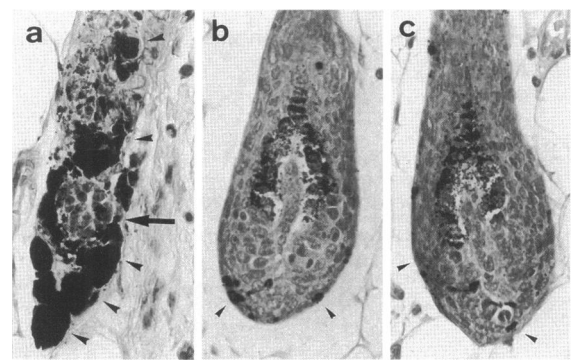

Figure 9. *CsA and FK 506 protect from CYP-induced follicle dystrophy.* **a:** *CYP-treated skin shows a representative back skin hair follicle with signs of advanced dystrophy (clumping of ectopic melanin granules* (black arrowheads) *and swelling of the dermal papilla* (black arrow)). *Giemsa; magnification, ×1000.* **b:** *Topical application of CsA (0.5%) to CYP-treated back skin results in the protection of follicles from massive signs of dystrophy. The follicle shows mild dystrophy with only single ectopic melanin granules* (black arrowheads). **c:** *FK 506 (0.5%) causes mitigation of follicle dystrophy after CYP treatment. The follicle shown here is only slightly dystrophic with hardly any ectopically located melanin granules* (black arrowheads).

topical agents unrelated to and/or less toxic than IPLs.[8] Comparing the murine hair growth effects of CsA, CsH, FK 506, and rapamycin *in vivo*, we have recently determined that the immunophilin-binding properties of these IPLs appear to be important, yet insufficient to induce anagen and inhibit catagen development in mice.[8] Instead, inhibition of the activity of the calcium/calmodulin-dependent phosphatase calcineurin may be a key signal transduction step in mediating hair cycle modulation by CsA and FK 506[19]; the IPL rapamycin, which does not inhibit calcineurin activity,[20,21] does not alter murine hair growth *in vivo*.[8] Therefore, we have proposed to directly inhibit calcineurin activity by appropriate antagonistic peptides[22–24] and/or to antagonize downstream events in the transcriptional control of recognized IPL target genes (cf Refs. 19 and 21), ideally by topical agents targeted to the hair follicle (cf Refs. 8 and 25).

Acknowledgments

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